# **Product Datasheet**

# ATP7b Antibody - BSA Free NB100-361

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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## NB100-361

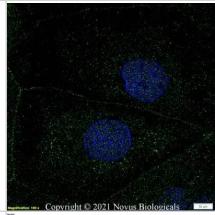
ATP7b Antibody - BSA Free

ATP7b Antibody - BSA Free	
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	165 kDa
Product Description	
Host	Rabbit
Gene ID	540
Gene Symbol	АТР7В
Species	Human, Rat, Mouse (Negative)
Reactivity Notes	Does not appear to react in mouse species.
Immunogen	A synthetic peptide made to an internal sequence near the C-terminus of human ATP7b. [UniProt# P35670]
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 1:1000, Immunocytochemistry/ Immunofluorescence 1:10-1:2000
Application Notes	This ATP7b antibody is useful for Immunocytochemistry/Immunofluorescence and Western blot. By WB, this antibody recognizes a band at 165 kDa,

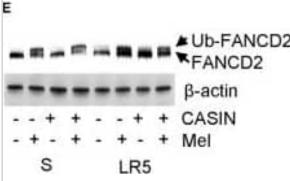
representing ATP7b. This antibody also recognizes a band at ~220 kDa. This antibody may work on endogenous protein in immunohistochemistry, but it has only been tested on transfected ovarian carcinoma cells at a 1:500 dilution. However, NB100-360 may demonstrate less background for this assay.

### **Images**

Immunocytochemistry/Immunofluorescence: ATP7b Antibody [NB100-361] - Caco-2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-ATP7b Antibody NB100-361 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



Western Blot: ATP7b Antibody [NB100-361] - Effects & mechanism of action of CASIN on melphalan-resistant MM cells. (A) CASIN reduces Cdc42 activity (Cdc42-GTP) but not Rac1 activity (Rac1-GTP). Melphalan-sensitive MM cells (S) were treated with Vehicle or CASIN (5 µM) for 8 h. Cdc42 & Rac1 activities were measured using pull-down assay. (B) CASIN (5 µM) inhibits proliferation of both S & melphalanresistant (LR5) MM cells. \*\*P < 0.01 vs. S-Vehicle; ##P < 0.01 vs. LR5-Vehicle. (C) CASIN (5 µM) preferentially causes apoptosis of melphalanresistant MM cells. S & LR5 cells were treated with or without CASIN or melphalan (Mel, 25 µM) or both for 2 days. Annexin V+ cells were analyzed using flow cytometry. \*\*P < 0.01. (D-F) Mechanism of action of CASIN on melphalan-resistant MM cells. (D) Cdc42 activity & expression are increased in LR5 cells compared to that in S cells. Cdc42 activity was measured using pull-down assay. β-Actin was used as loading control. Vertical lines indicate the gel lanes being switched in position from the original blots. (E) CASIN (5 µM) decreases melphalan-induced FANCD2 mono-ubiquitination (Ub-FANCD2) in LR5 but not in S cells. S & LR5 cells were treated with or without CASIN, Mel (25 μM), or both for 16 h. FANCD2 was detected using western blotting. β-Actin was used as loading control. (F) CASIN sensitizes melphalan-resistant but not sensitive MM cells to melphalan-induced DNA damage. y H2A histone family member X-positive (yH2AX+) cells were detected using flow cytometry. \*\*P < 0.01. Error bars represent means ± SD of triplicates & data are representative of three independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31632904), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



#### **Publications**

Nguyen P, Chakrabarti J, Li Y et al Rational Targeting of Cdc42 Overcomes Drug Resistance of Multiple Myeloma. Frontiers in Oncology 2019-10-01 [PMID: 31632904] (WB, Human)

Yoshizawa K, Nozaki S, Kitahara H et al. Copper efflux transporter (ATP7B) contributes to the acquisition of cisplatin-resistance in human oral squamous cell lines. Oncol Rep. 2007-10-01 [PMID: 17786364] (WB, Human)

Guo, Y et al. NH2-terminal signals in ATP7B Cu-ATPase mediate its Cu-dependent anterograde traffic in polarized hepatic cells. Am J Physiol Gastrointest Liver Physiol;289(5):G904-16. 2005-11-01 [PMID: 15994426] (WB, Rat, Human)



#### **Procedures**

## Immunohistochemistry protocol for ATP7b Antibody (NB100-361)

ATP7b Antibody:

Immunohistochemistry Procedure

Cell Preparation (At least 108 cells were used per block)

- 1. Harvesting cells:
- A. Trypsinization
- B. 15 minute centrifugation at 2,500 RPM
- C. PBS rinse
- D. 15 minute centrifugation at 2,500 RPM
- 2. Suspend cells in 10 ml of 10% formaldehyde in PBS, overnight @ RT.
- 3. Centrifuge cells at 2,500 RPM for 10 minutes.
- 4. Resuspend cells in 10 ml of 70% ethanol.
- 5. Centrifuge cells at 2,500 RPM and taken into 70% ethanol.

#### Cell Staining

- 1. Ribbon Thickness: 5 um
- 2. Deparaffination Agent: Xylin
- 3. Hydration: Ethanol in PBS
- 4. Blocking:
- A. endogeneous peroxidase: 0.3% H2O2 in PBS for 10 minutes
- B. endogeneous protein: 1% BSA for 20 minutes
- 6. Primary antibody, polyclonal anti-ATP7b (NB 100-361): 1:500, overnight @ 4 degrees Celcius
- 7. Secondary antibody, anti-rabbit (HRP): (dilute per manufacturer recommendation), 30 minutes @ RT
- 8. Wash 3x 15 minutes
- 9. Chromogen: AEC
- Counterstain: Mayers hematoxylin





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## **Products Related to NB100-361**

NBL1-07848 ATP7b Overexpression Lysate

HAF008 Goat anti-Rabbit IgG Secondary Antibody [HRP]

NB7160 Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]

NBP2-24891 Rabbit IgG Isotype Control

#### Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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